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# THE EFFECT OF MOUTHWASH ON ORAL MICROFLORA.

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## ABSTRACT.

Three different concentrations of mouthwashes 001, 002 and 003 formulated from $H_2O_2/Na_2B_4O_7$ were assayed for their inhibitory effect on *Streptococcus mutans*, *Streptococcus salivarius* and *Candida albicans* at different concentration of 12%, 9%, 6%, 3% and 1%. The results showed that the mouthwashes significantly reduced to different levels the microbial count of all organisms used. The reduction in microbial count ranged from initial count of 2.4 x $10^6$cfu/ml to 0.62 x $10^6$cfu/ml for *Streptococcus mutans*, 2.1 x $10^6$cfu/ml to 0.48 x $10^6$cfu/ml for *Streptococcus salivarius* and 1.0 x $10^6$cfu/ml to 0.23 x $10^6$cfu/ml for *Candida albicans*, depending on the mouthwash concentration. At $P< 0.05$, there was no significant difference ($P< 0.05$) amongst the three different mouthwashes in terms of inhibitory properties, though showing a significant decrease ($P< 0.05$) in microbial count. The mouthwashes contained the same compositional compounds but at different concentrations which most probably account for the observed differences in their inhibitory activities. The above results show that the mouthwashes 001, 002 and 003 have good antimicrobial properties.

KEYWORDS: Mouthwash, Streptococcal organisms, dental caries, periodontal diseases, Hygiene

## INTRODUCTION

The mouth is extensively colonized by a broad range of microbes. The mouth is also richly vascularised, making it susceptible to oral infection. The two most common types of oral infections, dental caries (decay) and the periodontal diseases (periodontitis and gingivitis) affect the tissues immediately adjacent to the teeth (Shay and Ship, 1995). The oral flora on the circulatory and respiratory system consists of a diverse and populous collection of bacteria, fungi, and transient viruses. Bacteria make up the largest number of varieties, more than 350 cultivable bacteria species have been identified in the mouth and molecular analyses suggest that an equal number of non- cultivable flora are also present (Samaranayake *et al*, 2002).

Dental caries is largely due to the colonization of the teeth by a group of Streptococcal organisms, although both Actinobacillus and Lactobacillus have been implicated as well. Dental caries is readily prevented through oral hygiene after the morning meal and prior to going to bed. Ideally, it is handled by dental assessment and professional cleaning on an annual or more frequent basis. Hygiene is accomplished through the use of one or more of variety of manual and/ or electrical toothbrushes and commercial toothpaste (Walinsky, 1994).

Gingivitis is a local oedematous reaction of the gum to exotoxins excreted by plaque organisms residing on the teeth. The affected tissues are usually asymptomatic and limited to the teeth on which plaque accumulated (Holm Pederson et al., 1975). Prevention of gingivitis is accomplished in the same manner and at the same time as prevention of dental caries. Periodontitis on the other hand is a lytic, inflammatory reaction of the plaque within the relatively anaerobic gingival sulcus. Microorganisms which are responsible for periodontitis are dominantly gram negative anaerobes (Nisengard *et al*., 1994). If the more aggressive cases of periodontitis are untreated, mobility and avulsion of teeth may eventually result (Nisengard *et al*., 1994). A periodontal abscess is an acute, focal, pyogenic inflammation that initiate within the gingival crevice or more commonly, the periodontal pocket, usually in response to the presence of some foreign body. The inflammatory reaction is exquisitely painful to palpation and chewing (Carbet, 2000).

In historical terms, oral hygiene as a means and an end is relatively novel concept which has only attracted the attention of researcher in recent times (Shay and Ship, 1995). Toothbrushes, tooth powder and paste were gradually embraced by the population at large, basically driven by purposeful advertising and the promise of

white teeth. Today, tooth brushing and other mechanical cleaning procedures are considered to be the most reliable means of controlling plaque, provided cleaning is sufficiently thorough and performed at regular

TABLE 1: TOTAL VIABLE COUNTS OF THE ISOLATES (CONTROL).

| ISOLATES | MICROBIAL COUNT (CFU/ml) |
|---|---|
| *Streptococcus mutans* | $2.4 \times 10^6$ |
| *Streptococcus salivarius* | $2.1 \times 10^6$ |
| *Candida albicans* | $1.0 \times 10^6$ |

TABLE 2: VIABLE COUNT OF *STREPTOCOCCUS MUTANS* TREATED WITH FIVE CONCENTRATIONS OF DIFFERENT TYPES OF MOUTHWASHES.

| ISOLATE | MOUTH WASH (mw) | CONCENTRATION OF MOUTHWASH (%) | PLATE COUNT PRE-TREATMENT (cfu/ml) | PLATE COUNT POST-TREATMENT (cfu/ ml) |
|---|---|---|---|---|
| *Streptococcus mutans* | MW 001 | 12 | $2.4 \times 10^6$ | $1.23 \times 10^6$ |
| | | 9 | $2.4 \times 10^6$ | $1.47 \times 10^6$ |
| | | 6 | $2.4 \times 10^6$ | $1.95 \times 10^6$ |
| | | 3 | $2.4 \times 10^6$ | $2.00 \times 10^6$ |
| | | 1 | $2.4 \times 10^6$ | $2.20 \times 10^6$ |
| | MW 002 | 12 | $2.4 \times 10^6$ | $1.46 \times 10^6$ |
| | | 9 | $2.4 \times 10^6$ | $1.73 \times 10^6$ |
| | | 6 | $2.4 \times 10^6$ | $1.84 \times 10^6$ |
| | | 3 | $2.4 \times 10^6$ | $1.81 \times 10^6$ |
| | | 1 | $2.4 \times 10^6$ | $1.90 \times 10^6$ |
| | MW 003 | 12 | $2.4 \times 10^6$ | $0.62 \times 10^6$ |
| | | 9 | $2.4 \times 10^6$ | $1.50 \times 10^6$ |
| | | 6 | $2.4 \times 10^6$ | $1.70 \times 10^6$ |
| | | 3 | $2.4 \times 10^6$ | $2.00 \times 10^6$ |
| | | 1 | $2.4 \times 10^6$ | $2.10 \times 10^6$ |

intervals (Cumming and Loe, 1973). Tooth brushing is paramount in maintaining good oral hygiene under the best of circumstances, however, toothbrushing is able to clean only the buccal lingual and occlusal surfaces (excluding pits and fissures); proximal and interdental area are essentially left untouched. Therefore, the target for modern hygiene programs or for any regimen attempting to prevent and reduce the incidence of caries and periodontal disease must put a major focus on the interdental and proximal areas of the dentition (Kinane, 1998). Mouthwashes (mouth rinses) are solutions or liquids used to rinse the mouth for a number of purposes. This include to remove or destroy bacteria, to act as an astringent, to deodorized and to have a therapeutic effect by relieving infection or preventing dental caries (Addy, 1986). A number of chemical agents are currently available in the market and are designed to assist individuals in their efforts to achieve and maintain oral health. While many agents are commercially available, the relative therapeutic benefits of most are not clearly defined (Kornman, 1986). Mouthwashes are manufactured in two forms: the wash and the spray. For most individuals the wash is simple and acceptable method for the delivery of topical medicaments into the oral cavity. Rinsing with a chlorhexidine mouthwash is arguably the most effective chemical method to date of controlling plaque accumulation (Kalaga *et al*, 1989). The most common regimen of use has been twice daily rinsing with 10ml of a 0.2% chlorhexidine solution (Kalaga *et al*, 1986; and Jenkins *et al*., 1988). However, with availability of more commercial mouthwashes similar antiplaque effects have been reported with twice daily rinsing with 15ml or 10ml of the solution according to manufacturer's direction of use (Leenstra *et al.*, 1996). Whereas previous studies have shown the ability of mouthwashes on plaque accumulation, plaque composition, either biochemical or microbiological, the possible effect of a mouthwash on bacterial load count in the mouth has received little or no attention in human studies in this environment, hence this work is aimed at determining the effect of mouthwash on oral microflora.

TABLE 3: VIABLE COUNT OF STREPTOCOCCUS SALIVARIUS TREATED WITH FIVE CONCENTRATIONS OF DIFFERENT TYPES OF MOUTHWASHES.

| ISOLATE | MOUTHWASH (MW) | CONCENTRATION OF MOUTHWASH (%) | PLATE COUNT PRE-TREATMENT (cfu/ml) | PLATE COUNT POST-TREATMENT (cfu/ml) |
|---|---|---|---|---|
| *Streptococcus salivarius* | MW 001 | 12 | 2.10 X $10^6$ | 1.10 X $10^6$ |
| | | 9 | 2.10 X $10^6$ | 1.30 X $10^6$ |
| | | 6 | 2.10 X $10^6$ | 1.76 X $10^6$ |
| | | 3 | 2.10 X $10^6$ | 1.87 X $10^6$ |
| | | 1 | 2.10 X $10^6$ | 1.93 X $10^6$ |
| | MW 002 | 12 | 2.10 X $10^6$ | 1.20 X $10^6$ |
| | | 9 | 2.10 X $10^6$ | 1.40 X $10^6$ |
| | | 6 | 2.10 X $10^6$ | 1.71 X $10^6$ |
| | | 3 | 2.10 X $10^6$ | 1.89 X $10^6$ |
| | | 1 | 2.10 X $10^6$ | 2.10 X $10^6$ |
| | MW 003 | 12 | 2.10 X $10^6$ | 0.48 X $10^6$ |
| | | 9 | 2.10 X $10^6$ | 0.96 X $10^6$ |
| | | 6 | 2.10 X $10^6$ | 1.10 X $10^6$ |
| | | 3 | 2.10 X $10^6$ | 1.48 X $10^6$ |
| | | 1 | 2.10 X $10^6$ | 2.10 X $10^6$ |

## MATERIALS AND METHODS.

EQUIPMENT/MATERIALS USED AND STERILIZATION METHODS: The equipment used include electronic balance, incubator, autoclave, reagents for biochemical analyses, conical flasks, slides and cover slides petridishes, test tubes, pipette, measuring cylinder, swab sticks, disinfectants (ethanol, formalin), bijoux bottle, hot air oven, mouthwash used labelled 001,002 and 003 formulated from $H_2O_2$⁄$Na_2B_4O_7$ (Nig. Pat. [2007]) (texture-liquid and colour- very clear), media (nutrient agar, blood agar, McConkey agar, nutrient broth and potato dextrose agar , peptone water). All glass wares were washed and sterilization was done at standard methods (160$^o$C for 1 hr). The incubation and hot oven used were also thoroughly cleaned and disinfected.

COLLECTION OF SAMPLES: Samples were collected from 36 students from Ambrose Alli University Ekpoma, taking into consideration the parameters (individual consent, full complement and good health of teeth, high standard of oral hygiene, having no medical history and not currently receiving pharmacotherapy) and with participant having had normal oral hygiene procedure(like tooth brushing with regular toothpaste) before collection of samples by rubbing gently around the gums, teeth, tongue and cervices of the mouth and transported immediately to the laboratory for microbiological investigation (Canfield and Griffen, 2000).

PREPARATION OF MOUTHWASH CONCENTRATION: Five concentrations of mouthwashes was prepared as follows: 12%, 9%, 6%, 3% and 1% of mouthwashes were prepared by mixing 12ml, 9ml, 6ml and 1ml respectively in 100ml of distilled water.

SERIAL DILUTION AND PLATE COUNT: 1ml of stock microbial solution was put in 9ml of sterile distilled water and shaken vigorously. 1ml of the supernatant was pipette into 9ml of sterile distilled water. This was serially diluted from $10^{-1}$ to $10^{-10}$. 1ml of the diluents was pipette into 10 sterile petri dishes and sterile nutrient agar was poured, rocked and allowed to gel before incubating at 37$^o$C for 24hrs.

IDENTIFICATION OF ISOLATES: The isolates were identified on the basis of their cultural characteristics (colonial morphological), gram staining and other biochemical reactions.

DETERMINATION OF EFFECT OF MOUTHWASHES ON THE ORAL MICROFLORA AND STATISTICAL ANALYSIS: This was determined in terms of the effect they had in the reduction or elimination of the oral micro flora identified by a comparison of control and experimental isolate count. Results obtained were statistically analyzed using the t-test method.

TABLE 4: VIABLE COUNT OF CANDIDA ALBICANS TREATED WITH FIVE CONCENTRATIONS OF DIFFERENT TYPES OF MOUTHWASHES.

| ISOLATE | MOUTHWASH (MW) | CONCENTRATION OF MOUTHWASH (%) | PLATE COUNT PRE- TREATMENT (cfu/ml) | PLATE COUNT POST-TREATMENT (cfu/ml) |
|---|---|---|---|---|
| *Candida albicans* | MW 001 | 12<br>9<br>6<br>3<br>1 | 1.0 X $10^6$<br>1.0 X $10^6$<br>1.0 X $10^6$<br>1.0 X $10^6$<br>1.0 X $10^6$ | 0.23 X $10^6$<br>0.62 X $10^6$<br>0.70 X $10^6$<br>0.85 X $10^6$<br>0.97 X $10^6$ |
| | MW 002 | 12<br>9<br>6<br>3<br>1 | 1.0 X $10^6$<br>1.0 X $10^6$<br>1.0 X $10^6$<br>1.0 X $10^6$<br>1.0 X $10^6$ | 0.36 X $10^6$<br>0.48 X $10^6$<br>0.64 X $10^6$<br>0.87 X $10^6$<br>0.93 X $10^6$ |
| | MW 003 | 12<br>9<br>6<br>3<br>1 | 1.0 X $10^6$<br>1.0 X $10^6$<br>1.0 X $10^6$<br>1.0 X $10^6$<br>1.0 X $10^6$ | 0.96 X $10^6$<br>0.78 X $10^6$<br>0.63 X $10^6$<br>0.57 X $10^6$<br>0,50 X $10^6$ |

## RESULTS.

All the mouthwashes used showed varying degree of inhibition on the isolated microorganisms. Treatment with each mouthwash reduced the number of colony forming units (cfu) of the viable organisms. The total viable counts of the isolates prior to treatment with mouthwashes (control) is shown on table 1 while tables 2, 3 and 4 show the microbial counts of the isolates after treatment with different concentration of the mouthwashes. The isolated organisms include *Streptococcus salivarius, Streptocuccos mutans* and *Candida albicans.*

## DISCUSSION

The oral microorganisms yielded specifically by all 36 swabs include *Streptococcus mutans*, *Streptococcus salivarius* and *Candida albicans.* The three different mouth washes had inhibitory effect on microorganisms with varying degrees depending on their concentration. It was observed that at higher concentrations, the different mouthwashes had more effect on the microorganisms. Mouthwash 003 had more inhibitory effect on the bacterial organisms (*Streptococcus mutans* and *Streptococcus salivarius*) when compared with mouthwashes 001 and 002. The effect of the mouthwashes were observed to produce inhibition on the microbial load as there were decrease from initial microbial load count after the use of the mouthwashes. However, at lower concentration, mouthwash 003 had inhibitory effect on the fungi organism (*Candida albicans*) but at higher concentration it effect on this organism was observed to decrease. This suggests that low concentration is required to effect inhibitory action against fungal and higher concentration is required for antibacterial activities. This finding agreed with Combe (1980) and Ashley *et al.*, (1998).

Inside *Streptococcus mutans*, the range of decrease of micro flora was 1.23 x $10^6$ - 2.20 x $10^6$ cfu/ml, 1.46 x $10^6$ - 1.9 x $10^6$ cfu/ml and 0.62 x $10^6$ - 2.1 x $10^6$ cfu/ml for mouthwash 001, 002 and 003 respectively when compared to the original counts before treatments which was 2.4 x $10^6$ cfu/ml (Table 2). In the case of *Streptococcus salivarius*, the decrease range was from 1.10 x $10^6$ - 1.93 x $10^6$cfu/ml, 1.20 x $10^6$ - 2.01 x $10^6$cfu/ml and 0.48 x $10^6$ - 2.1 x $10^6$ cfu/ml for mouthwash 001, 002 and 003 when compared to the pre-mouthwash count which was 2.10 x $10^6$ cfu/ml (Table 3). However, in the case of *Candida albicans* the initial count of 1.0 x $10^6$cfu/ml but when treated with different concentrations of mouthwashes 001, 002 and 003, the microbial counts ranges from 0.20 x $10^6$ - 0.96 x $10^6$ cfu/ml, 0.36 x $10^6$ - 0.93 x $10^6$ cfu/ml and 0.50 x $10^6$ - 0.96 x $10^6$ cfu/ml respectively (Table 4).

This finding shows that mouthwashes are useful for purposes of removing or destroying bacteria from the mouth and having a therapeutic effect. Also the differences observed in the inhibitory effect of mouthwash shows that a

different component which have different mode of action against microbes and that the type of mouthwash used determines the extent of microbial growth inhibition.

As result of the statistical analysis of data at $P < 0.05$, there was a significant reduction in microbial count after treatment with mouthwash compared to the initial microbial count before treatment. However, there was no significant difference ($P < 0.05$) among the three different mouthwashes in the way they separately reduced microbial count.

In conclusion, we recommended that mouthwashes should be developed to have broad spectrum antibiotics in order to enhance their effectiveness, also that further investigation should be carried out on the various mouthwashes used in this study in order to determine and improve on the antimicrobial active constituents present that give the mouthwashes it inhibitory property observed in them and also invivo studies using laboratory animals should be carried out to determine the safety of the mouthwashes before use in human.

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